



**HAL**  
open science

## Linkage disequilibrium between the allele and wildtype and alleles: identification of haplotypes in healthy Nordic populations

Rasmus S. Pedersen, Charlotte Brasch-Andersen, Sarah C. Sim, Troels K. Bergmann, Jónrit Halling, Maria S. Petersen, Pál Weihe, Hege Edvardsen, Vessela N. Kristensen, Kim Brøsen, et al.

### ► To cite this version:

Rasmus S. Pedersen, Charlotte Brasch-Andersen, Sarah C. Sim, Troels K. Bergmann, Jónrit Halling, et al.. Linkage disequilibrium between the allele and wildtype and alleles: identification of haplotypes in healthy Nordic populations. *European Journal of Clinical Pharmacology*, 2010, 66 (12), pp.1199-1205. 10.1007/s00228-010-0864-8 . hal-00612005

**HAL Id: hal-00612005**

**<https://hal.science/hal-00612005>**

Submitted on 28 Jul 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.


L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



**Linkage disequilibrium between the rapid CYP2C19\*17 allele and wildtype CYP2C8 and CYP2C9 alleles: Identification of CYP2C haplotypes in healthy Nordic populations**

Journal:	<i>European Journal of Clinical Pharmacology</i>
Manuscript ID:	EJCP-2010-0210.R1
Type of submission:	Original
Date Submitted by the Author:	01-Jul-2010
Complete List of Authors:	<p>Pedersen, Rasmus; University of Southern Denmark, Clinical Pharmacology                  Brasch-Andersen, Charlotte; University of Southern Denmark, Clinical Pharmacology                  Sim, Sarah; Karolinska Institutet, Section of Pharmacogenetics, Department of Pharmacology and Physiology                  Bergmann, Troels; University of Southern Denmark, Clinical Pharmacology                  Halling, Jónrit; The Faroese Hospital System, Department of Occupational Medicines and Public Health                  Petersen, Maria; The Faroese Hospital System, Department of Occupational Medicines and Public Health                  Weihe, Pál; The Faroese Hospital System, Department of Occupational Medicines and Public Health                  Edvardsen, Hege; Oslo University Hospital, Radiumhospitalet, Department of Genetics                  Kristensen, Vessela; Oslo University Hospital, Radiumhospitalet, Department of Genetics                  Brosen, Kim; University of Southern Denmark, Clinical Pharmacology                  Ingelman-Sundberg, Magnus; Karolinska Institutet, Section of Pharmacogenetics, Department of Pharmacology and Physiology</p>

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

  
**Linkage disequilibrium between the *CYP2C19\*17* allele and  
wildtype *CYP2C8* and *CYP2C9* alleles: Identification of  
*CYP2C* haplotypes in healthy Nordic populations**

Comment [RP1]: The word  
"rapid" has been removed from the  
Title

Rasmus S. Pedersen<sup>1,2</sup>, Charlotte Brasch-Andersen<sup>1</sup> Sarah C. Sim<sup>2</sup>, Troels  
K. Bergmann<sup>1</sup>, Jónrit Halling<sup>1,3</sup>, Maria S. Petersen<sup>1,3</sup>, Pál Weihe<sup>3</sup>, Hege  
Edvardsen<sup>4,5</sup>, Vessela N. Kristensen<sup>4,5,6</sup>, Kim Brøsen<sup>1</sup> and Magnus  
Ingelman-Sundberg<sup>2</sup>

<sup>1</sup>Institute of Public Health, Clinical Pharmacology, University of Southern Denmark,  
Odense, Denmark

<sup>2</sup>Section of Pharmacogenetics, Department of Physiology and Pharmacology,  
Karolinska Institutet, Stockholm, Sweden

<sup>3</sup>Department of Occupational Medicines and Public Health, The Faroese Hospital  
System, Tórshavn, Faroe Islands

<sup>4</sup>Department of Genetics, Institute of Cancer Research, Oslo University Hospital  
Radiumhospitalet, Oslo, Norway

<sup>5</sup>Institute of Clinical Medicine, University of Oslo, Oslo, Norway

<sup>6</sup>EpiGen, Institute for Clinical Medicine, Akershus University Hospital, Oslo, Norway

Correspondence:

Rasmus Steen Pedersen, Institute of Public Health, Clinical Pharmacology, University  
of Southern Denmark, Odense, Denmark. E-mail: [rpetersen@health.sdu.dk](mailto:rpetersen@health.sdu.dk). Phone:  
+45 65503305. Fax: +45 65916089

**Abstract**

*Purpose.* To determine the distribution of clinically important *CYP2C* genotypes and allele frequencies in healthy Nordic populations with special focus on linkage disequilibrium.

*Methods.* 896 healthy subjects from three Nordic populations (Faroese, Danish and Norwegian) were genotyped for five frequent and clinically important *CYP2C* allelic variants: the defective *CYP2C8\*3*, *CYP2C9\*2*, *CYP2C9\*3* and *CYP2C19\*2* alleles, and the *CYP2C19\*17* allele that causes rapid drug metabolism. Linkage disequilibrium was evaluated and *CYP2C* haplotypes were inferred in the entire population.

*Results.* Ten *CYP2C* haplotypes were inferred of which the most frequent (49%) was the *CYP2C* wildtype haplotype carrying *CYP2C8\*1*, *CYP2C9\*1* and *CYP2C19\*1*. The second most frequent haplotype (19%) is composed by *CYP2C19\*17*, *CYP2C8\*1* and *CYP2C9\*1*. This predicted haplotype accounts for 99.7% of the *CYP2C19\*17* alleles found in the 896 subjects.

*Conclusion.* *CYP2C19\*17* is a frequent genetic variant in Nordic populations that exists in strong linkage disequilibrium with wild type *CYP2C8\*1* and *CYP2C9\*1* alleles, which effectively makes it a determinant for a haplotype exhibiting efficient *CYP2C* substrate metabolism.

## Introduction

Human cytochrome P450 2C (CYP2C) enzymes are responsible for the metabolism of approximately 25% of clinically used drugs [1] and some endogenous substances such as arachidonic acid [2]. The human CYP2C subfamily includes CYP2C8, CYP2C9, CYP2C18 and CYP2C19 that are homologous and share more than 80% amino acid sequence identity [3]. However, only CYP2C8, CYP2C9 and CYP2C19 are considered of clinical importance [4], since CYP2C18 mRNA is detected at relatively low amounts [5]. The entire *CYP2C* gene locus spans approximately 500 kb on chromosome 10 with the gene order *Cen-2C18-2C19-2C9-2C8-Tel* (Figure 1) and each of the genes consist of nine exons with conserved exon/intron boundaries [6]. CYP2C8, CYP2C9 and CYP2C19 exhibit clinically important genetic polymorphism as a result of single nucleotide polymorphisms (SNPs) ([www.cypalleles.ki.se](http://www.cypalleles.ki.se)).

CYP2C8 catalyzes the metabolism of several clinically used drugs such as the anti-cancer drug paclitaxel, the anti-arrhythmic drug amiodarone, the antidiabetic drug repaglinide and the thiazolidinediones rosiglitazone and troglitazone [7].

Fourteen allelic variants of CYP2C8 have been described of which some cause poor metabolism [8]. *CYP2C8\*3* is a quantitatively and functionally relevant variant allele in the Caucasian population with a reported frequency of 10-23% but rare or absent in Asian and African populations [5]. *CYP2C8\*3* is defined by two amino acid substitutions in exon 3 and exon 8 (Table 1), respectively, causing decreased paclitaxel turnover [9] and decreased paclitaxel clearance *in vivo* [10].

CYP2C9 is among the most important drug metabolizing enzymes in humans. It is involved in the metabolism of several important drugs including phenytoin, losartan, (*S*)-warfarin, tolbutamide and numerous NSAIDs [11]. Of the 34 allelic variants that have been described [12], the two clinically most important variant alleles are *CYP2C9\*2* and *CYP2C9\*3*, which both cause a defective CYP2C9 enzyme (Table 1)[13]. The allele frequencies of *CYP2C9\*2* and *CYP2C9\*3* are approximately 10-15% and 5-10%, respectively, in European populations [14].

CYP2C19 is another important drug metabolizing enzyme encoded by a highly polymorphic gene [15]. The racemic anticonvulsant drug mephenytoin is the classic probe drug where the 4-hydroxylation of the (*S*)-enantiomer is catalysed by CYP2C19. Furthermore, a range of clinically important drugs including diazepam,

1  
2 omeprazole and other proton pump inhibitors, the antidepressants citalopram,  
3 escitalopram and imipramine, the antimalarial drugs proguanil and chlorproguanil, the  
4  $\beta$ -adrenoceptor blocker propranolol, and the antiplatelet drug clopidogrel are  
5 metabolized or bioactivated by CYP2C19. Population studies have shown that  
6 individuals that are homozygous for the inactivating allele *CYP2C19\*2* that leads to a  
7 splicing defect (Table 1), are phenotypic poor metabolizers of CYP2C19 substrates  
8 [5,16,17]. The CYP2C19 poor metabolizers only represent 3-5% of Caucasian  
9 populations, but is found in 12-23% of Asian populations [3]. In recent years, much  
10 attention has focused on the allele *CYP2C19\*17* that correlates with high *in vivo*  
11 CYP2C19 activity and is present at an allele frequency of 18% in a Swedish healthy  
12 population [18]. The initial finding of a rapid drug metabolism phenotype was later  
13 supported by a clear association of lower plasma concentration of omeprazole and  
14 escitalopram in subjects homozygous for *CYP2C19\*17* [19,20]. Interestingly,  
15 *CYP2C19\*17* also leads to increased bioactivation of the antiplatelet drug clopidogrel  
16 that causes an almost 4-fold higher risk of bleeding complications in *CYP2C19\*17*  
17 homozygotes, but no beneficial antithrombotic effect for patients undergoing  
18 percutaneous coronary intervention [21]. As opposed to subjects homozygous for the  
19 *CYP2C19\*1* allele, subjects homozygous for *CYP2C19\*17* display a very low  
20 interindividual variation in CYP2C19 metabolism [19,22]. The rapid phenotype  
21 caused by *CYP2C19\*17* has been explained by a recruitment of transcription factor(s)  
22 by the -806C>T conversion (Table 1) in the gene promoter region that causes an  
23 increased transcriptional rate [18]. A hypothesis is that *CYP2C19\*17* is also a  
24 haplotype marker within the *CYP2C* locus [19]. In fact, haplotype block structures at  
25 the *CYP2C* locus have been described [23,24]. Almost all subjects carrying  
26 *CYP2C8\*3* also carry *CYP2C9\*2* and three quarters of all subjects carrying  
27 *CYP2C9\*2* also carry *CYP2C8\*3* [25]. *CYP2C19\*2* and *CYP2C19\*3* has also been  
28 shown to be linked with *CYP2C18* mutations in Japanese subjects [26].  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43

44 The aim of this study was to determine important *CYP2C* genotypes with  
45 special focus on *CYP2C19\*17* in healthy Nordic populations, and to investigate the  
46 linkage disequilibrium between them. |  
47  
48  
49  
50

**Comment [RP2]:** Detailed info about the allelic variant has been moved to Table 1

## 51 Methods

52  
53  
54  
55  
56  
57  
58  
59  
60

### Samples

DNA samples from 276 healthy volunteers living around Odense in Denmark and previously genotyped for *CYP2C9\*2* and *CYP2C9\*3* [14], and 311 Faroese samples from healthy volunteers previously genotyped for *CYP2C8\*3*, *CYP2C9\*2*, *CYP2C9\*3* and *CYP2C19\*2* [27] were included in this study. Furthermore, DNA samples from 328 Norwegian Caucasian blood donors that had not been genotyped for *CYP2C* variants were included [28,29].

### Genotyping

A total of 915 DNA samples from three different populations were available for genotyping and the aim was to cover the five clinically important *CYP2C* allelic variants; *CYP2C8\**, *CYP2C9\*2*, *CYP2C9\*3*, *CYP2C19\*2* and *CYP2C19\*17*. All 276 DNA samples from Denmark [14] were successfully genotyped for *CYP2C8\*3*, *CYP2C19\*2* and *CYP2C19\*17* and all the 311 Faroese samples were successfully genotyped for *CYP2C19\*17*. Of the 328 Norwegian DNA samples, 309 were successfully genotyped for all five *CYP2C* variants. The analyses were performed using Applied Biosystems TaqMan® SNP genotyping assays (Table 1) according to the guidelines and conducted on an Applied Biosystems StepOne Plus apparatus or an Applied Biosystems 7500 Real Time PCR apparatus.

**Comment [RP3]:** Info of the assays is presented in Table 1

### Statistics

Possible deviation from Hardy Weinberg equilibrium was tested for each SNP with Pearson's Chi-Square test with a level of significance of 5%. Overall differences of allele frequencies between the populations were also tested by Chi-Squared test with a level of significance of 5%. "Head to head" comparison between the populations was tested with Fisher's exact test with a level of significance of 5%.

### Linkage disequilibrium and haplotype inference

The software Haploview was used to visualize the structure of pair-wise linkage disequilibrium (LD) between the SNPs (in terms of Lewontin's  $D'$ ) [30]. Individual haplotypes were inferred by use of the five *CYP2C* SNPs and software package PHASE version 2.1 [31,32]. This software infers haplotypes from population genotype data using a Bayesian statistical method. We performed PHASE analyses

1  
2 with default settings ten times with different random seed. Additionally, haplotype  
3 frequencies were estimated using the expectation maximization algorithm (EM) in  
4 STATA10 (StataCorp, Texas, USA).  
5  
6  
7  
8  
9

## 10 Results

11 We performed genotyping of *CYP2C8\*3*, *CYP2C9\*2*, *CYP2C9\*3*, *CYP2C19\*2* and  
12 *CYP2C19\*17* in three different Nordic populations. The genotype and allele  
13 frequencies in the Danish, Faroese and Norwegian samples are presented in Table 2.  
14 All SNPs in all three populations were in Hardy-Weinberg equilibrium displaying  $\chi^2$ -  
15 values less than 3.84.  
16  
17  
18  
19

20 An overall significant difference in allele frequency of *CYP2C19\*17* was  
21 found between the populations ( $P=0.010$ ). The allele frequency of 15 % in the Faroese  
22 population ( $n=311$ ) was lower than the 20% in the Danish population ( $n=276$ )  
23 ( $P=0.038$ ) and the 22% in the Norwegian population ( $n=309$ ,  $P=0.003$ ). No  
24 statistically significant difference between the Danish and the Norwegian population  
25 was found. None of the four other SNPs displayed significantly different allele  
26 frequencies between the populations.  
27  
28  
29  
30

31 When analyzing all three populations, none of the 40 subjects homozygous for  
32 the rapid *CYP2C19\*17* allele carried the defective *CYP2C8\*3*, *CYP2C9\*2* or  
33 *CYP2C9\*3* alleles. In addition, none of the 9 subjects homozygous for *CYP2C8\*3*  
34 carried *CYP2C19\*17* and none of the 20 subjects homozygous for *CYP2C9\*2* and/or  
35 *CYP2C9\*3* carried *CYP2C19\*17*.  
36  
37  
38  
39

40 The linkage disequilibrium between the five analysed SNPs as based on  
41 Haploview calculations is visualized in Figure 2. The calculations show complete LD  
42 ( $D'=1$ ) between *CYP2C19\*17* and the four other allelic variants. Complete linkage  
43 disequilibrium was also observed between *CYP2C19\*2* and the two *CYP2C9* alleles  
44 *CYP2C9\*2* and *CYP2C9\*3*. The analysis also displays strong linkage disequilibrium  
45 between *CYP2C9\*2* and *CYP2C8\*3* ( $D'=0.92$ ).  
46  
47  
48  
49

50 Ten *CYP2C* haplotypes were inferred (Table 3). The *CYP2C* haplotype  
51 frequencies predicted by PHASE and STATA were almost identical. Six of the  
52  
53  
54  
55  
56  
57  
58  
59  
60



haplotypes (No.1-5 and 9) account for 99% of the haplotypes. The most frequent haplotype (No. 1) with an inferred frequency of 49% carries the *CYP2C8\*1*, *CYP2C9\*1* and *CYP2C19\*1* alleles. The second most frequent haplotype (No. 2) with an inferred frequency of 19% is composed by *CYP2C19\*17*, *CYP2C8\*1* and *CYP2C9\*1* wildtype alleles, and is representing 99.7% (342 out of 343) of all predicted *CYP2C19\*17*-containing haplotypes. In one Norwegian subject, a single variant haplotype with *CYP2C19\*17* in combination with *CYP2C8\*3* was inferred, thus leading to an estimated haplotype frequency of <0.001 in the total material of 896 subjects (Table 2). The previously reported linkage disequilibrium between *CYP2C9\*2* and *CYP2C8\*3* presented by Yasar *et al.* [25] was also supported by our data. In detail, 94% (144 out of 154) of the haplotypes with *CYP2C8\*3* were also predicted to carry *CYP2C9\*2*, and 79% (144 out of 183) of the haplotypes with *CYP2C9\*2* were also predicted to carry *CYP2C8\*3*.

## Discussion

This study provides support to the hypothesis of a linkage disequilibrium between *CYP2C19\*17* and functional *CYP2C* alleles. This hypothesis was first expressed by Baldwin *et al.* [19] but was based on relatively limited data. Further indications can be found by reviewing the genotype information presented by Rudberg *et al.* [22]. In the present study, we found that *CYP2C19\*17* is predicted to be almost exclusively present together with wild type alleles of *CYP2C8* and *CYP2C9*, thus mediating rapid metabolism of *CYP2C19* substrates and normal (mostly referred to as extensive) metabolism of *CYP2C8* and *CYP2C9* substrates.

The prediction of one haplotype out of the 343 *CYP2C19\*17*-containing haplotypes that exists in combination with *CYP2C8\*3* must be recognized. However the existence of these two variants on separate haplotypes is still a theoretic possibility, although predicted to be rare. Nevertheless, the clinical picture is clear showing that subjects with *CYP2C19\*17* that confers a rapid metabolism of *CYP2C19* substrates, are likely to display extensive metabolic capacity toward other *CYP2C* substrates.

This study also confirms the linkage between *CYP2C9\*2* and *CYP2C8\*3* with similar

1  
2  
3 correlation percentages as previously shown by Yasar *et al.* [25]. Thus, poor  
4 metabolisers of CYP2C9 substrate drugs are also likely to poorly metabolise CYP2C8  
5 substrates. It is interesting to note that across the three genes CYP2C19, CYP2C9 and  
6 CYP2C8, the five SNPs examined occur in six distinct haplotypes 99% of the time  
7 and further that these haplotypes exclusively carry none or just a single SNP except  
8 for haplotype No.9 characterized by two SNPs. This is in accordance with the notion  
9 that SNPs are introduced to the genome as a single mutation occurring at a single  
10 point in time in a single individual and vertically propagated to the population. In  
11 future pharmacogenetic studies it might be worthwhile to consider these haplotypes as  
12 an alternative to the individual SNPs as this approach might offer a more powerful  
13 design that can possibly reveal effects of interplay between *cis* variants, which is  
14 impossible in simple analysis of individual SNPs.

20 The current study supports the general assumption that the Nordic populations  
21 have a relatively similar distribution of cytochrome P450 genotypes. The  
22 *CYP2C19\*17* allele was first reported in Swedes with a frequency of 18% [18], but a  
23 recent study report a Swedish allele frequency of 20% [33]. The Norwegian  
24 *CYP2C19\*17* allele frequency of 22% in the current study of healthy volunteers is  
25 identical with the reported frequency in 166 Norwegian psychiatric patients [22], and  
26 similar to that observed in the Danish subjects of the present study (20%). The  
27 Faroese population had a statistically significant lower allele frequency of 15%, but  
28 no correction for multiple testing was done. A similar allele frequency of 20% was  
29 found in Greek population [34] and in African populations: 18% in Ethiopians [18]  
30 and 17% in Ugandans [35]. In Asian populations very low frequencies of  
31 *CYP2C19\*17* has been reported: 4% in Chinese [18], 1% in Japanese [36] and 0.3%  
32 in Koreans [33]. On the other hand, the frequency is apparently higher in Central  
33 Europe: 25% in a German breast cancer population [37] and 28% in a population of  
34 Polish peptic ulcer patients [38]. Overall, *CYP2C19\*17* must be considered prevalent  
35 in all the Nordic populations studied here, and the frequency of subjects homozygous  
36 for *CYP2C19\*17* only ranged from 3.5 to 5.1 % in the studied material.

48 In conclusion, we have in the current study identified a common haplotype in  
49 the Nordic population carrying the *CYP2C19\*17*, *CYP2C8\*1* and *CYP2C9\*1* alleles,  
50 thus representing a haplotype encoding efficient metabolism of all drugs that are  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Comment [RP4]:** More info of  
*CYP2C19\*17* allele freq in other  
populations

1  
2 substrates for CYP2C enzymes. The *CYP2C19\*17* genotype could thus be used as a  
3  
4 determinant for extensive metabolism of CYP2C substrates in Nordic subjects.  
5

#### 6 **Acknowledgements**

7  
8 This research was supported in part by grants from The Swedish Research Council,  
9  
10 Torsten and Ragnar Söderbergs Stiftelser, Karolinska Institutet, The Danish Research  
11  
12 Council for Health and Disease, and The Lundbeck Foundation. We thank Pernille  
13  
14 Jordan for analytical expertise.  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

**References**

1. Ingelman-Sundberg M (2004) Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. *Naunyn Schmiedebergs Arch Pharmacol* 369:89-104
2. Niwa T, Murayama N, Yamazaki H (2009) Oxidation of Endobiotics Mediated by Xenobiotic-metabolizing Forms of Human Cytochrome P450. *Curr Drug Metab* 10:700-712
3. Goldstein JA, de Morais SM (1994) Biochemistry and molecular biology of the human CYP2C subfamily. *Pharmacogenetics* 4:285-99
4. Goldstein JA (2001) Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. *Br J Clin Pharmacol* 52:349-55
5. Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. (2007) Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoeigenetic and clinical aspects. *Pharmacol Ther* 116:496-526.
6. Romkes M, Faletto MB, Blaisdell JA, Raucy JL, Goldstein JA (1991) Cloning and expression of complementary DNAs for multiple members of the human cytochrome P450IIC subfamily. *Biochemistry* 30:3247-55
7. Totah RA, Rettie AE (2005) Cytochrome P450 2C8: substrates, inhibitors, pharmacogenetics, and clinical relevance. *Clin Pharmacol Ther* 77:341-52.
8. <http://www.cypalleles.ki.se/cyp2c8.htm> assessed in June 2010
9. Dai D, Zeldin DC, Blaisdell JA, Chanas B, Coulter SJ, Ghanayem BI, Goldstein JA (2001) Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. *Pharmacogenetics* 11:597-607
10. Bergmann TK, Vach W, Gréen H, Karlsson MO, Friberg L, Nielsen F, Pedersen RS, Mirza MR, Brasch-Andersen C, Brosen K (2010) Impact of CYP2C8\*3 on paclitaxel clearance: a population pharmacokinetic and pharmacogenomic study in 93 patients with ovarian cancer. *Pharmacogenomics J* (April 6, Epub ahead of print)
11. Miners JO, Birkett DJ (1998) Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. *Br J Clin Pharmacol* 45:525-38
12. <http://www.cypalleles.ki.se/cyp2c9.htm> assessed in June 2010

13. King BP, Khan TI, Aithal GP, Kamali F, Daly AK (2004) Upstream and coding region CYP2C9 polymorphisms: correlation with warfarin dose and metabolism. *Pharmacogenetics* 14:813-22.
14. Pedersen RS, Verstuyft C, Becquemont L, Jaillon P, Brøsen K (2004) Cytochrome P4502C9 (*CYP2C9*) genotypes in a Nordic population in Denmark. *Basic Clin Pharmacol Toxicol* 94:151-152
15. <http://www.cypalleles.ki.se/cyp2c19.htm> assessed in June 2010
16. Wilkinson GR, Guengerich FP, Branch RA (1989) Genetic polymorphism of S-mephenytoin hydroxylation. *Pharmacol Ther* 43:53-76
17. de Morais SM, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA (1994) The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. *J Biol Chem* 269:15419-22
18. Sim SC, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, Ingelman-Sundberg M (2006) A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther* 79:103-13
19. Baldwin RM, Ohlsson S, Pedersen RS, Mwinyi J, Ingelman-Sundberg M, Eliasson E, Bertilsson L (2008) Increased omeprazole metabolism in carriers of the CYP2C19\*17 allele; a pharmacokinetic study in healthy volunteers. *Br J Clin Pharmacol* 65:767-74
20. Ohlsson Rosenberg S, Mwinyi J, Andersson M, Baldwin RM, Pedersen RS, Sim SC, Bertilsson L, Ingelman-Sundberg M, Eliasson (2008) Kinetics of omeprazole and escitalopram in relation to the CYP2C19\*17 allele in healthy subjects. *Eur J Clin Pharmacol* 64:1175-9
21. Sibbing D, Koch W, Gebhard D, Schuster T, Braun S, Stegherr J, Morath T, Schömig A, von Beckerath N, Kastrati A. (2010) Cytochrome 2C19\*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. *Circulation.*;121:512-8.
22. Rudberg I, Mohebi B, Hermann M, Refsum H, Molden E (2008) Impact of the ultrarapid CYP2C19\*17 allele on serum concentration of escitalopram in psychiatric patients. *Clin Pharmacol Ther* 83:322-7
23. Ahmadi KR, Weale ME, Xue ZY, Soranzo N, Yarnall DP, Briley JD, Maruyama Y, Kobayashi M, Wood NW, Spurr NK, Burns DK, Roses AD, Saunders AM,

- 1  
2 Goldstein DB (2005) A single-nucleotide polymorphism tagging set for human  
3 drug metabolism and transport. *Nat Genet* 37:84-9  
4  
5 24. Walton R, Kimber M, Rockett K, Trafford C, Kwiatkowski D, Sirugo G (2005)  
6 Haplotype block structure of the cytochrome P450 CYP2C gene cluster on  
7 chromosome 10. *Nat Genet* 37:915-6  
8  
9 25. Yasar U, Lundgren S, Eliasson E, Bennet A, Wiman B, de Faire U, Rane A (2002)  
10 Linkage between the CYP2C8 and CYP2C9 genetic polymorphisms. *Biochem*  
11 *Biophys Res Commun* 299:25-8  
12  
13 26. Mamiya K, Ieiri I, Miyahara S, Imai J, Furuumi H, Fukumaki Y, Ninomiya H,  
14 Tashiro N, Yamada H, Higuchi S (1998) Association of polymorphisms in the  
15 cytochrome P450 (CYP) 2C19 and 2C18 genes in Japanese epileptic patients.  
16 *Pharmacogenetics* 8:87-90  
17  
18 27. Halling J, Petersen MS, Damkier P, Nielsen F, Grandjean P, Weihe Pál, Lundgren  
19 S, Lundblad MS, Brøsen K (2005) Polymorphisms of CYP2D6, CYP2C19,  
20 CYP2C9, CYP2C8 in the Faroese population. *Eur J Clin Pharmacol* 61:491-497  
21  
22 28. Heimdal K, Andersen TI, Skrede M, Fosså SD, Berg K, Børresen AL (1995)  
23 Association studies of estrogen receptor polymorphisms in a Norwegian testicular  
24 cancer population. *Cancer Epidemiol Biomarkers Prev* 4(2):123-6  
25  
26 29. Tefre T, Daly AK, Armstrong M, Leathart JB, Idle JR, Brøgger A, Børresen AL  
27 (1994) Genotyping of the CYP2D6 gene in Norwegian lung cancer patients and  
28 controls. *Pharmacogenetics* 4(2):47-57  
29  
30 30. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization  
31 of LD and haplotype maps. *Bioinformatics* 21:263-265  
32  
33 31. Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype  
34 reconstruction from population data. *Am J Hum Genet* 68: 978-989  
35  
36 32. Stephens M, Scheet P (2005) Accounting for decay of linkage disequilibrium in  
37 haplotype inference and missing-data imputation. *Am J Hum Genet* 76:449-462  
38  
39 33. Ramsjö M, Aklillu E, Bohman L, Ingelman-Sundberg M, Roh HK, Bertilsson L  
40 (2010) CYP2C19 activity comparison between Swedes and Koreans: effect of  
41 genotype, sex, oral contraceptive use, and smoking. *Eur J Clin Pharmacol* (Epub  
42 ahead of print)  
43  
44  
45  
46  
47  
48  
49  
50



- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
34. [Ragia G, Arvanitidis KI, Tavridou A, Manolopoulos VG \(2009\) Need for reassessment of reported CYP2C19 allele frequencies in various populations in view of CYP2C19\\*17 discovery: the case of Greece. Pharmacogenomics 10:43-9.](#)
  35. [Miura J, Obua C, Abbo C, Kaneko S, Tateishi T \(2009\) Cytochrome P450 2C19 genetic polymorphisms in Ugandans. Eur J Clin Pharmacol 65: 319-20](#)
  36. [Sugimoto K, Uno T, Yamazaki H, Tateishi T \(2008\) Limited frequency of the CYP2C19\\*17 allele and its minor role in a Japanese population. Br J Clin Pharmacol 65:437-439.](#)
  37. [Justenhoven C, Hamann U, Pierl CB, Baisch C, Harth V, Rabstein S, Spickenheuer A, Pesch B, Brüning T, Winter S, Ko YD, Brauch H \(2009\) CYP2C19\\*17 is associated with decreased breast cancer risk. Breast Cancer Res Treat 115:391-6](#)
  38. [Gawrońska-Szklarz B, Siuda A, Kurzawski M, Bielicki D, Marlicz W, Drożdżik M \(2010\) Effects of CYP2C19, MDR1, and interleukin 1-B gene variants on the eradication rate of Helicobacter pylori infection by triple therapy with pantoprazole, amoxicillin, and metronidazole. Eur J Clin Pharmacol 66:681-7](#)

Comment [RP5]: Six more ref.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22

## Linkage disequilibrium between the *CYP2C19\*17* allele and wildtype *CYP2C8* and *CYP2C9* alleles: Identification of *CYP2C* haplotypes in healthy Nordic populations

23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48

Rasmus S. Pedersen<sup>1,2</sup>, Charlotte Brasch-Andersen<sup>1</sup> Sarah C. Sim<sup>2</sup>, Troels  
K. Bergmann<sup>1</sup>, Jónrit Halling<sup>1,3</sup>, Maria S. Petersen<sup>1,3</sup>, Pál Weihe<sup>3</sup>, Hege  
Edvardsen<sup>4,5</sup>, Vessela N. Kristensen<sup>4,5,6</sup>, Kim Brøsen<sup>1</sup> and Magnus  
Ingelman-Sundberg<sup>2</sup>

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

<sup>1</sup>Institute of Public Health, Clinical Pharmacology, University of Southern Denmark,  
Odense, Denmark

<sup>2</sup>Section of Pharmacogenetics, Department of Physiology and Pharmacology,  
Karolinska Institutet, Stockholm, Sweden

<sup>3</sup>Department of Occupational Medicines and Public Health, The Faroese Hospital  
System, Tórshavn, Faroe Islands

<sup>4</sup>Department of Genetics, Institute of Cancer Research, Oslo University Hospital  
Radiumhospitalet, Oslo, Norway

<sup>5</sup>Institute of Clinical Medicine, University of Oslo, Oslo, Norway

<sup>6</sup>EpiGen, Institute for Clinical Medicine, Akershus University Hospital, Oslo, Norway

Correspondence:

Rasmus Steen Pedersen, Institute of Public Health, Clinical Pharmacology, University  
of Southern Denmark, Odense, Denmark. E-mail: [rpedersen@health.sdu.dk](mailto:rpedersen@health.sdu.dk). Phone:  
+45 65503305. Fax: +45 65916089



**Abstract**

*Purpose.* To determine the distribution of clinically important *CYP2C* genotypes and allele frequencies in healthy Nordic populations with special focus on linkage disequilibrium.

*Methods.* 896 healthy subjects from three Nordic populations (Faroese, Danish and Norwegian) were genotyped for five frequent and clinically important *CYP2C* allelic variants: the defective *CYP2C8\*3*, *CYP2C9\*2*, *CYP2C9\*3* and *CYP2C19\*2* alleles, and the *CYP2C19\*17* allele that causes rapid drug metabolism. Linkage disequilibrium was evaluated and *CYP2C* haplotypes were inferred in the entire population.

*Results.* Ten *CYP2C* haplotypes were inferred of which the most frequent (49%) was the *CYP2C* wildtype haplotype carrying *CYP2C8\*1*, *CYP2C9\*1* and *CYP2C19\*1*. The second most frequent haplotype (19%) is composed by *CYP2C19\*17*, *CYP2C8\*1* and *CYP2C9\*1*. This predicted haplotype accounts for 99.7% of the *CYP2C19\*17* alleles found in the 896 subjects.

*Conclusion.* *CYP2C19\*17* is a frequent genetic variant in Nordic populations that exists in strong linkage disequilibrium with wild type *CYP2C8\*1* and *CYP2C9\*1* alleles, which effectively makes it a determinant for a haplotype exhibiting efficient *CYP2C* substrate metabolism.

## Introduction

Human cytochrome P450 2C (CYP2C) enzymes are responsible for the metabolism of approximately 25% of clinically used drugs [1] and some endogenous substances such as arachidonic acid [2]. The human CYP2C subfamily includes CYP2C8, CYP2C9, CYP2C18 and CYP2C19 that are homologous and share more than 80% amino acid sequence identity [3]. However, only CYP2C8, CYP2C9 and CYP2C19 are considered of clinical importance [4], since CYP2C18 mRNA is detected at relatively low amounts [5]. The entire *CYP2C* gene locus spans approximately 500 kb on chromosome 10 with the gene order Cen-2C18-2C19-2C9-2C8-Tel (Figure 1) and each of the genes consist of nine exons with conserved exon/intron boundaries [6]. CYP2C8, CYP2C9 and CYP2C19 exhibit clinically important genetic polymorphism as a result of single nucleotide polymorphisms (SNPs) ([www.cypalleles.ki.se](http://www.cypalleles.ki.se)).

CYP2C8 catalyzes the metabolism of several clinically used drugs such as the anti-cancer drug paclitaxel, the anti-arrhythmic drug amiodarone, the antidiabetic drug repaglinide, and the thiazolidinediones rosiglitazone and troglitazone [7]. Fourteen allelic variants of CYP2C8 have been described of which some cause poor metabolism [8]. *CYP2C8\*3* is a quantitatively and functionally relevant variant allele in the Caucasian population with a reported frequency of 10-23% but rare or absent in Asian and African populations [5]. *CYP2C8\*3* is defined by two amino acid substitutions in exon 3 and exon 8 (Table 1), respectively, causing decreased paclitaxel turnover [9] and decreased paclitaxel clearance *in vivo* [10].

CYP2C9 is among the most important drug metabolizing enzymes in humans. It is involved in the metabolism of several important drugs including phenytoin, losartan, (*S*)-warfarin, tolbutamide and numerous NSAIDs [11]. Of the 34 allelic variants that have been described [12], the two clinically most important variant alleles are *CYP2C9\*2* and *CYP2C9\*3*, which both cause a defective CYP2C9 enzyme (Table 1)[13]. The allele frequencies of *CYP2C9\*2* and *CYP2C9\*3* are approximately 10-15% and 5-10%, respectively, in European populations [14].

CYP2C19 is another important drug metabolizing enzyme encoded by a highly polymorphic gene [15]. The racemic anticonvulsant drug mephenytoin is the classic probe drug where the 4-hydroxylation of the (*S*)-enantiomer is catalysed by CYP2C19. Furthermore, a range of clinically important drugs including diazepam,

1  
2  
3 omeprazole and other proton pump inhibitors, the antidepressants citalopram,  
4 escitalopram and imipramine, the antimalarial drugs proguanil and chlorproguanil, the  
5  $\beta$ -adrenoceptor blocker propranolol, and the antiplatelet drug clopidogrel are  
6 metabolized or bioactivated by CYP2C19. Population studies have shown that  
7 individuals that are homozygous for the inactivating allele *CYP2C19\*2* that leads to a  
8 splicing defect (Table 1), are phenotypic poor metabolizers of CYP2C19 substrates  
9 [5,16,17]. The CYP2C19 poor metabolizers only represent 3-5% of Caucasian  
10 populations, but is found in 12-23% of Asian populations [3]. In recent years, much  
11 attention has focused on the allele *CYP2C19\*17* that correlates with high *in vivo*  
12 CYP2C19 activity and is present at an allele frequency of 18% in a Swedish healthy  
13 population [18]. The initial finding of a rapid drug metabolism phenotype was later  
14 supported by a clear association of lower plasma concentration of omeprazole and  
15 escitalopram in subjects homozygous for *CYP2C19\*17* [19,20]. Interestingly,  
16 *CYP2C19\*17* also leads to increased bioactivation of the antiplatelet drug clopidogrel  
17 that causes an almost 4-fold higher risk of bleeding complications in *CYP2C19\*17*  
18 homozygotes, but no beneficial antithrombotic effect for patients undergoing  
19 percutaneous coronary intervention [21]. As opposed to subjects homozygous for the  
20 *CYP2C19\*1* allele, subjects homozygous for *CYP2C19\*17* display a very low  
21 interindividual variation in CYP2C19 metabolism [19,22]. The rapid phenotype  
22 caused by *CYP2C19\*17* has been explained by a recruitment of transcription factor(s)  
23 by the -806C>T conversion (Table 1) in the gene promoter region that causes an  
24 increased transcriptional rate [18]. A hypothesis is that *CYP2C19\*17* is also a  
25 haplotype marker within the *CYP2C* locus [19]. In fact, haplotype block structures at  
26 the *CYP2C* locus have been described [23,24]. Almost all subjects carrying  
27 *CYP2C8\*3* also carry *CYP2C9\*2* and three quarters of all subjects carrying  
28 *CYP2C9\*2* also carry *CYP2C8\*3* [25]. *CYP2C19\*2* and *CYP2C19\*3* has also been  
29 shown to be linked with *CYP2C18* mutations in Japanese subjects [26].  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50

51  
52 The aim of this study was to determine important *CYP2C* genotypes with  
53 special focus on *CYP2C19\*17* in healthy Nordic populations, and to investigate the  
54 linkage disequilibrium between them.  
55  
56  
57  
58  
59  
60

## Methods

### *Samples*

DNA samples from 276 healthy volunteers living around Odense in Denmark and previously genotyped for *CYP2C9\*2* and *CYP2C9\*3* [14], and 311 Faroese samples from healthy volunteers previously genotyped for *CYP2C8\*3*, *CYP2C9\*2*, *CYP2C9\*3* and *CYP2C19\*2* [27] were included in this study. Furthermore, DNA samples from 328 Norwegian Caucasian blood donors that had not been genotyped for *CYP2C* variants were included [28,29].

### *Genotyping*

A total of 915 DNA samples from three different populations were available for genotyping and the aim was to cover the five clinically important *CYP2C* allelic variants; *CYP2C8\**, *CYP2C9\*2*, *CYP2C9\*3*, *CYP2C19\*2* and *CYP2C19\*17*. All 276 DNA samples from Denmark [14] were successfully genotyped for *CYP2C8\*3*, *CYP2C19\*2* and *CYP2C19\*17* and all the 311 Faroese samples were successfully genotyped for *CYP2C19\*17*. Of the 328 Norwegian DNA samples, 309 were successfully genotyped for all five *CYP2C* variants. The analyses were performed using Applied Biosystems TaqMan® SNP genotyping assays (Table 1) according to the guidelines and conducted on an Applied Biosystems StepOne Plus apparatus or an Applied Biosystems 7500 Real Time PCR apparatus.

### *Statistics*

Possible deviation from Hardy Weinberg equilibrium was tested for each SNP with Pearsons Chi-Square test with a level of significance of 5%. Overall differences of allele frequencies between the populations were also tested by Chi-Squared test with a level of significance of 5%. “Head to head” comparison between the populations was tested with Fisher’s exact test with a level of significance of 5%.

### *Linkage disequilibrium and haplotype inference*

The software Haploview was used to visualize the structure of pair-wise linkage disequilibrium (LD) between the SNPs (in terms of Lewontin's D') [30]. Individual haplotypes were inferred by use of the five *CYP2C* SNPs and software package PHASE version 2.1 [31,32]. This software infers haplotypes from population genotype data using a Bayesian statistical method. We performed PHASE analyses

1  
2  
3 with default settings ten times with different random seed. Additionally, haplotype  
4 frequencies were estimated using the expectation maximization algorithm (EM) in  
5 STATA10 (StataCorp, Texas, USA).  
6  
7  
8  
9

## 10 11 12 **Results**

13  
14 We performed genotyping of *CYP2C8\*3*, *CYP2C9\*2*, *CYP2C9\*3*, *CYP2C19\*2* and  
15 *CYP2C19\*17* in three different Nordic populations. The genotype and allele  
16 frequencies in the Danish, Faroese and Norwegian samples are presented in Table 2.  
17 All SNPs in all three populations were in Hardy-Weinberg equilibrium displaying  $\chi^2$ -  
18 values less than 3.84.  
19  
20  
21  
22  
23

24 An overall significant difference in allele frequency of *CYP2C19\*17* was  
25 found between the populations ( $P=0.010$ ). The allele frequency of 15 % in the Faroese  
26 population ( $n=311$ ) was lower than the 20% in the Danish population ( $n=276$ )  
27 ( $P=0.038$ ) and the 22% in the Norwegian population ( $n=309$ ,  $P=0.003$ ). No  
28 statistically significant difference between the Danish and the Norwegian population  
29 was found. None of the four other SNPs displayed significantly different allele  
30 frequencies between the populations.  
31  
32  
33  
34  
35  
36  
37

38 When analyzing all three populations, none of the 40 subjects homozygous for  
39 the rapid *CYP2C19\*17* allele carried the defective *CYP2C8\*3*, *CYP2C9\*2* or  
40 *CYP2C9\*3* alleles. In addition, none of the 9 subjects homozygous for *CYP2C8\*3*  
41 carried *CYP2C19\*17* and none of the 20 subjects homozygous for *CYP2C9\*2* and/or  
42 *CYP2C9\*3* carried *CYP2C19\*17*.  
43  
44  
45  
46  
47

48 The linkage disequilibrium between the five analysed SNPs as based on  
49 Haploview calculations is visualized in Figure 2. The calculations show complete LD  
50 ( $D'=1$ ) between *CYP2C19\*17* and the four other allelic variants. Complete linkage  
51 disequilibrium was also observed between *CYP2C19\*2* and the two *CYP2C9* alleles  
52 *CYP2C9\*2* and *CYP2C9\*3*. The analysis also displays strong linkage disequilibrium  
53 between *CYP2C9\*2* and *CYP2C8\*3* ( $D'=0.92$ ).  
54  
55  
56  
57  
58

59 Ten *CYP2C* haplotypes were inferred (Table 3). The *CYP2C* haplotype  
60 frequencies predicted by PHASE and STATA were almost identical. Six of the

haplotypes (No.1-5 and 9) account for 99% of the haplotypes. The most frequent haplotype (No. 1) with an inferred frequency of 49% carries the *CYP2C8\*1*, *CYP2C9\*1* and *CYP2C19\*1* alleles. The second most frequent haplotype (No. 2) with an inferred frequency of 19% is composed by *CYP2C19\*17*, *CYP2C8\*1* and *CYP2C9\*1* wildtype alleles, and is representing 99.7% (342 out of 343) of all predicted *CYP2C19\*17*-containing haplotypes. In one Norwegian subject, a single variant haplotype with *CYP2C19\*17* in combination with *CYP2C8\*3* was inferred, thus leading to an estimated haplotype frequency of <0.001 in the total material of 896 subjects (Table 2). The previously reported linkage disequilibrium between *CYP2C9\*2* and *CYP2C8\*3* presented by Yasar *et al.* [25] was also supported by our data. In detail, 94% (144 out of 154) of the haplotypes with *CYP2C8\*3* were also predicted to carry *CYP2C9\*2*, and 79% (144 out of 183) of the haplotypes with *CYP2C9\*2* were also predicted to carry *CYP2C8\*3*.

## Discussion

This study provides support to the hypothesis of a linkage disequilibrium between *CYP2C19\*17* and functional *CYP2C* alleles. This hypothesis was first expressed by Baldwin *et al.* [19] but was based on relatively limited data. Further indications can be found by reviewing the genotype information presented by Rudberg *et al.* [22]. In the present study, we found that *CYP2C19\*17* is predicted to be almost exclusively present together with wild type alleles of *CYP2C8* and *CYP2C9*, thus mediating rapid metabolism of *CYP2C19* substrates and normal (mostly referred to as extensive) metabolism of *CYP2C8* and *CYP2C9* substrates.

The prediction of one haplotype out of the 343 *CYP2C19\*17*-containing haplotypes that exists in combination with *CYP2C8\*3* must be recognized. However the existence of these two variants on separate haplotypes is still a theoretic possibility, although predicted to be rare. Nevertheless, the clinical picture is clear showing that subjects with *CYP2C19\*17* that confers a rapid metabolism of *CYP2C19* substrates, are likely to display extensive metabolic capacity toward other *CYP2C* substrates.

This study also confirms the linkage between *CYP2C9\*2* and *CYP2C8\*3* with similar



1  
2  
3 correlation percentages as previously shown by Yasar *et al.* [25]. Thus, poor  
4 metabolisers of CYP2C9 substrate drugs are also likely to poorly metabolise CYP2C8  
5 substrates. It is interesting to note that across the three genes CYP2C19, CYP2C9 and  
6 CYP2C8, the five SNPs examined occur in six distinct haplotypes 99% of the time  
7 and further that these haplotypes exclusively carry none or just a single SNP except  
8 for haplotype No.9 characterized by two SNPs. This is in accordance with the notion  
9 that SNPs are introduced to the genome as a single mutation occurring at a single  
10 point in time in a single individual and vertically propagated to the population. In  
11 future pharmacogenetic studies it might be worthwhile to consider these haplotypes as  
12 an alternative to the individual SNPs as this approach might offer a more powerful  
13 design that can possibly reveal effects of interplay between *cis* variants, which is  
14 impossible in simple analysis of individual SNPs.  
15

16  
17 The current study supports the general assumption that the Nordic populations  
18 have a relatively similar distribution of cytochrome P450 genotypes. The  
19 *CYP2C19\*17* allele was first reported in Swedes with a frequency of 18% [18], but a  
20 recent study report a Swedish allele frequency of 20% [33]. The Norwegian  
21 *CYP2C19\*17* allele frequency of 22% in the current study of healthy volunteers is  
22 identical with the reported frequency in 166 Norwegian psychiatric patients [22], and  
23 similar to that observed in the Danish subjects of the present study (20%). The  
24 Faroese population had a statistically significant lower allele frequency of 15%, but  
25 no correction for multiple testing was done. A similar allele frequency of 20% was  
26 found in Greek population [34] and in African populations: 18% in Ethiopians [18]  
27 and 17% in Ugandans [35]. In Asian populations very low frequencies of  
28 *CYP2C19\*17* has been reported: 4% in Chinese [18], 1% in Japanese [36] and 0.3%  
29 in Koreans [33]. On the other hand, the frequency is apparently higher in Central  
30 Europe; 25% in a German breast cancer population [37] and 28% in a population of  
31 Polish peptic ulcer patients [38]. Overall, *CYP2C19\*17* must be considered prevalent  
32 in all the Nordic populations studied here, and the frequency of subjects homozygous  
33 for *CYP2C19\*17* only ranged from 3.5 to 5.1 % in the studied material.  
34  
35

36  
37 In conclusion, we have in the current study identified a common haplotype in  
38 the Nordic population carrying the *CYP2C19\*17*, *CYP2C8\*1* and *CYP2C9\*1* alleles,  
39 thus representing a haplotype encoding efficient metabolism of all drugs that are  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 substrates for CYP2C enzymes. The *CYP2C19\*17* genotype could thus be used as a  
4  
5 determinant for extensive metabolism of CYP2C substrates in Nordic subjects.  
6  
7

### 8 **Acknowledgements**

9  
10 This research was supported in part by grants from The Swedish Research Council,  
11  
12 Torsten and Ragnar Söderbergs Stiftelser, Karolinska Institutet, The Danish Research  
13  
14 Council for Health and Disease, and The Lundbeck Foundation. We thank Pernille  
15  
16 Jordan for analytical expertise.  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review



## References

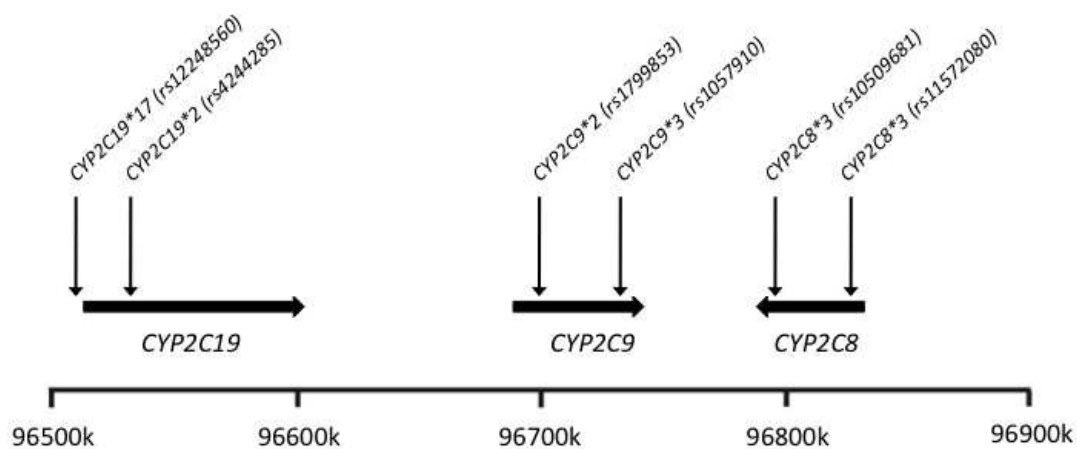
1. Ingelman-Sundberg M (2004) Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. *Naunyn Schmiedebergs Arch Pharmacol* 369:89-104
2. Niwa T, Murayama N, Yamazaki H (2009) Oxidation of Endobiotics Mediated by Xenobiotic-metabolizing Forms of Human Cytochrome P450. *Curr Drug Metab* 10:700-712
3. Goldstein JA, de Morais SM (1994) Biochemistry and molecular biology of the human CYP2C subfamily. *Pharmacogenetics* 4:285-99
4. Goldstein JA (2001) Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. *Br J Clin Pharmacol* 52:349-55
5. Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. (2007) Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. *Pharmacol Ther* 116:496-526.
6. Romkes M, Faletto MB, Blaisdell JA, Raucy JL, Goldstein JA (1991) Cloning and expression of complementary DNAs for multiple members of the human cytochrome P450IIC subfamily. *Biochemistry* 30:3247-55
7. Totah RA, Rettie AE (2005) Cytochrome P450 2C8: substrates, inhibitors, pharmacogenetics, and clinical relevance. *Clin Pharmacol Ther* 77:341-52.
8. <http://www.cypalleles.ki.se/cyp2c8.htm> assessed in June 2010
9. Dai D, Zeldin DC, Blaisdell JA, Chanas B, Coulter SJ, Ghanayem BI, Goldstein JA (2001) Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. *Pharmacogenetics* 11:597-607
10. Bergmann TK, Vach W, Gréen H, Karlsson MO, Friberg L, Nielsen F, Pedersen RS, Mirza MR, Brasch-Andersen C, Brosen K (2010) Impact of CYP2C8\*3 on paclitaxel clearance: a population pharmacokinetic and pharmacogenomic study in 93 patients with ovarian cancer. *Pharmacogenomics J* (April 6, Epub ahead of print)
11. Miners JO, Birkett DJ (1998) Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. *Br J Clin Pharmacol* 45:525-38
12. <http://www.cypalleles.ki.se/cyp2c9.htm> assessed in June 2010

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
13. King BP, Khan TI, Aithal GP, Kamali F, Daly AK (2004) Upstream and coding region CYP2C9 polymorphisms: correlation with warfarin dose and metabolism. *Pharmacogenetics* 14:813-22.
14. Pedersen RS, Verstuyft C, Becquemont L, Jaillon P, Brøsen K (2004) Cytochrome P4502C9 (*CYP2C9*) genotypes in a Nordic population in Denmark. *Basic Clin Pharmacol Toxicol* 94:151-152
15. <http://www.cypalleles.ki.se/cyp2c19.htm> assessed in June 2010
16. Wilkinson GR, Guengerich FP, Branch RA (1989) Genetic polymorphism of S-mephenytoin hydroxylation. *Pharmacol Ther* 43:53-76
17. de Morais SM, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA (1994) The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. *J Biol Chem* 269:15419-22
18. Sim SC, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, Ingelman-Sundberg M (2006) A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther* 79:103-13
19. Baldwin RM, Ohlsson S, Pedersen RS, Mwinyi J, Ingelman-Sundberg M, Eliasson E, Bertilsson L (2008) Increased omeprazole metabolism in carriers of the CYP2C19\*17 allele; a pharmacokinetic study in healthy volunteers. *Br J Clin Pharmacol* 65:767-74
20. Ohlsson Rosenberg S, Mwinyi J, Andersson M, Baldwin RM, Pedersen RS, Sim SC, Bertilsson L, Ingelman-Sundberg M, Eliasson (2008) Kinetics of omeprazole and escitalopram in relation to the CYP2C19\*17 allele in healthy subjects. *Eur J Clin Pharmacol* 64:1175-9
21. Sibbing D, Koch W, Gebhard D, Schuster T, Braun S, Stegherr J, Morath T, Schömig A, von Beckerath N, Kastrati A. (2010) Cytochrome 2C19\*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. *Circulation.*;121:512-8.
22. Rudberg I, Mohebi B, Hermann M, Refsum H, Molden E (2008) Impact of the ultrarapid CYP2C19\*17 allele on serum concentration of escitalopram in psychiatric patients. *Clin Pharmacol Ther* 83:322-7
23. Ahmadi KR, Weale ME, Xue ZY, Soranzo N, Yarnall DP, Briley JD, Maruyama Y, Kobayashi M, Wood NW, Spurr NK, Burns DK, Roses AD, Saunders AM,

- 1  
2  
3 Goldstein DB (2005) A single-nucleotide polymorphism tagging set for human  
4 drug metabolism and transport. *Nat Genet* 37:84-9  
5  
6  
7 24. Walton R, Kimber M, Rockett K, Trafford C, Kwiatkowski D, Sirugo G (2005)  
8 Haplotype block structure of the cytochrome P450 CYP2C gene cluster on  
9 chromosome 10. *Nat Genet* 37:915-6  
10  
11  
12 25. Yasar U, Lundgren S, Eliasson E, Bennet A, Wiman B, de Faire U, Rane A (2002)  
13 Linkage between the CYP2C8 and CYP2C9 genetic polymorphisms. *Biochem*  
14 *Biophys Res Commun* 299:25-8  
15  
16  
17 26. Mamiya K, Ieiri I, Miyahara S, Imai J, Furuumi H, Fukumaki Y, Ninomiya H,  
18 Tashiro N, Yamada H, Higuchi S (1998) Association of polymorphisms in the  
19 cytochrome P450 (CYP) 2C19 and 2C18 genes in Japanese epileptic patients.  
20 *Pharmacogenetics* 8:87-90  
21  
22  
23 27. Halling J, Petersen MS, Damkier P, Nielsen F, Grandjean P, Weihe Pál, Lundgren  
24 S, Lundblad MS, Brøsen K (2005) Polymorphisms of CYP2D6, CYP2C19,  
25 CYP2C9, CYP2C8 in the Faroese population. *Eur J Clin Pharmacol* 61:491-497  
26  
27  
28 28. Heimdal K, Andersen TI, Skrede M, Fosså SD, Berg K, Børresen AL (1995)  
29 Association studies of estrogen receptor polymorphisms in a Norwegian testicular  
30 cancer population. *Cancer Epidemiol Biomarkers Prev* 4(2):123-6  
31  
32  
33 29. Tefre T, Daly AK, Armstrong M, Leathart JB, Idle JR, Brøgger A, Børresen AL  
34 (1994) Genotyping of the CYP2D6 gene in Norwegian lung cancer patients and  
35 controls. *Pharmacogenetics* 4(2):47-57  
36  
37  
38 30. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization  
39 of LD and haplotype maps. *Bioinformatics* 21:263-265  
40  
41  
42 31. Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype  
43 reconstruction from population data. *Am J Hum Genet* 68: 978-989  
44  
45  
46 32. Stephens M, Scheet P (2005) Accounting for decay of linkage disequilibrium in  
47 haplotype inference and missing-data imputation. *Am J Hum Genet* 76:449-462  
48  
49  
50 33. Ramsjö M, Aklillu E, Bohman L, Ingelman-Sundberg M, Roh HK, Bertilsson L  
51 (2010) CYP2C19 activity comparison between Swedes and Koreans: effect of  
52 genotype, sex, oral contraceptive use, and smoking. *Eur J Clin Pharmacol* (Epub  
53 ahead of print)  
54  
55  
56  
57  
58  
59  
60

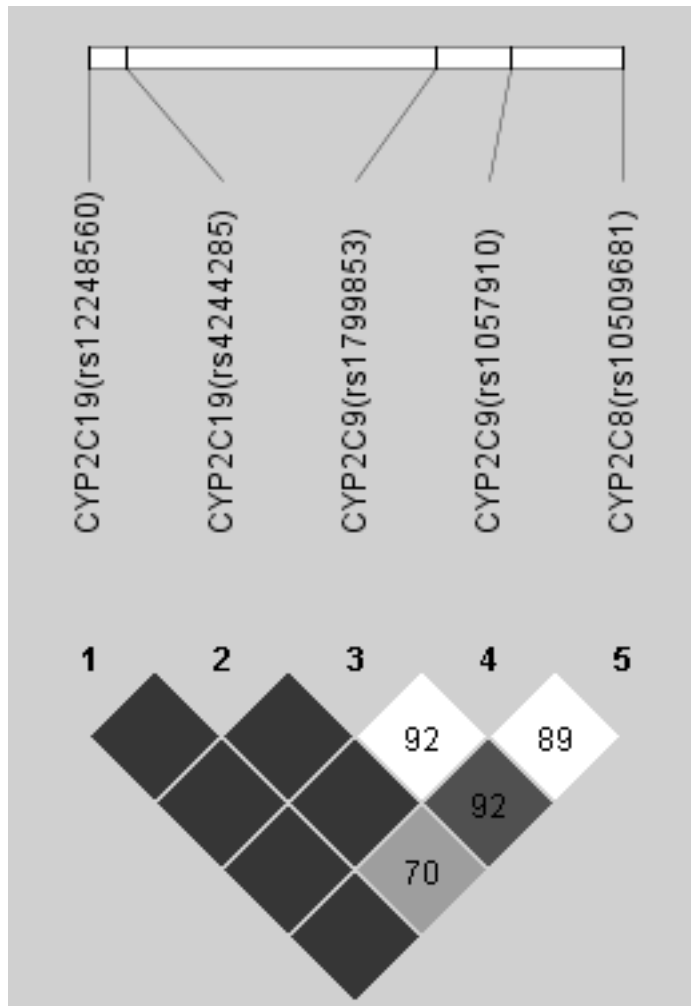
- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
34. Ragia G, Arvanitidis KI, Tavridou A, Manolopoulos VG (2009) Need for reassessment of reported CYP2C19 allele frequencies in various populations in view of CYP2C19\*17 discovery: the case of Greece. *Pharmacogenomics* 10:43-9.
35. Miura J, Obua C, Abbo C, Kaneko S, Tateishi T (2009) Cytochrome P450 2C19 genetic polymorphisms in Ugandans. *Eur J Clin Pharmacol* 65: 319-20
36. Sugimoto K, Uno T, Yamazaki H, Tateishi T (2008) Limited frequency of the CYP2C19\*17 allele and its minor role in a Japanese population. *Br J Clin Pharmacol* 65:437-439.
37. Justenhoven C, Hamann U, Pierl CB, Baisch C, Harth V, Rabstein S, Spickenheuer A, Pesch B, Brüning T, Winter S, Ko YD, Brauch H (2009) CYP2C19\*17 is associated with decreased breast cancer risk. *Breast Cancer Res Treat* 115:391-6
38. Gawrońska-Szklarz B, Siuda A, Kurzawski M, Bielicki D, Marlicz W, Drożdżik M (2010) Effects of CYP2C19, MDR1, and interleukin 1-B gene variants on the eradication rate of *Helicobacter pylori* infection by triple therapy with pantoprazole, amoxicillin, and metronidazole. *Eur J Clin Pharmacol* 66:681-7

Figure 1



The organisation of *CYP2C19*, *CYP2C9*, and *CYP2C8* genes on chromosome 10 as based on the human genome map found at [www.ncbi.nlm.nih \(build 36.3\)](http://www.ncbi.nlm.nih.gov/build36.3). Thin arrows indicate the position of the allelic variants analyzed. Thick arrows represent the genes studied indicating the direction of gene transcription.

Figure 2



The linkage disequilibrium (LD) map of the 5 SNPs analysed in the current study was created using Haploview. The dark squares without numbers show complete LD ( $D'=1$ ,  $LOD > 3.6$ ).

**Table 1**The studied *CYP2C* allelic variants

<b>CYP allele</b>	<b>rs number</b>	<b>Gene nucleotide change</b>	<b>Protein Effect</b>	<b>TaqMan® Assay Number</b>
<i>CYP2C19*17</i>	rs12248560	-806C>T	Ile <sub>331</sub> →Val	C__469857_10
<i>CYP2C19*2</i>	rs4244285	19154G>A	Splicing defect	C__25986767_70
<i>CYP2C9*2</i>	rs1799853	3608C>T	Arg <sub>144</sub> →Lys	C__25986767_10
<i>CYP2C9*3</i>	rs1057910	42614A>C	Ile <sub>359</sub> →Leu	C__27104892_10
<i>CYP2C8*3</i>	rs10509681	40411A>G	Lys <sub>399</sub> →Arg	C__25625782_20
<i>CYP2C8*3</i>	rs11572080	2130G>A	Arg <sub>139</sub> →Lys	Not tested

**Table 2**

Genotype and allele frequencies of *CYP2C8*, *CYP2C9* and *CYP2C19* allelic SNPs in Danish, Faroese and Norwegian subjects.

	<i>Danish</i>		<i>Faroese</i>		<i>Norwegian</i>	
<i>CYP2C8</i>	Number of subjects	Frequency	Number of subjects	Frequency	Number of subjects	Frequency
<i>CYP2C8</i> *1/*1	226	0.819	268	0.862	257	0.832
<i>CYP2C8</i> *1/*3	45	0.163	43	0.138	48	0.155
<i>CYP2C8</i> *3/*3	5	0.018	0	0.000	4	0.013
<i>Allele</i>	Number of alleles	Frequency	Number of alleles	Frequency	Number of alleles	Frequency
<i>CYP2C8</i> *1	497	0.900	579	0.931	562	0.909
<i>CYP2C8</i> *3	55	0.100	43	0.069	56	0.091
<i>CYP2C9</i>	Number of subjects	Frequency	Number of subjects	Frequency	Number of subjects	Frequency
<i>CYP2C9</i> *1/*1	190	0.688	228	0.733	213	0.689
<i>CYP2C9</i> *1/*2	53	0.192	50	0.161	55	0.178
<i>CYP2C9</i> *1/*3	23	0.083	28	0.090	36	0.117
<i>CYP2C9</i> *2/*2	4	0.014	0	0.000	2	0.007
<i>CYP2C9</i> *3/*3	0	0.000	0	0.000	1	0.003
<i>CYP2C9</i> *2/*3	6	0.022	5	0.016	2	0.007
<i>Allele</i>	Number of alleles	Frequency	Number of alleles	Frequency	Number of alleles	Frequency
<i>CYP2C9</i> *1	456	0.826	534	0.859	517	0.837
<i>CYP2C9</i> *2	67	0.121	55	0.088	61	0.099
<i>CYP2C9</i> *3	29	0.053	33	0.053	40	0.065
<i>CYP2C19</i>	Number of subjects	Frequency	Number of subjects	Frequency	Number of subjects	Frequency
<i>CYP2C19</i> *1/*1	122	0.442	143	0.460	122	0.395
<i>CYP2C19</i> *1/*2	51	0.185	73	0.235	62	0.201
<i>CYP2C19</i> *1/*17	63	0.228	51	0.164	82	0.265
<i>CYP2C19</i> *2/*2	6	0.022	10	0.032	4	0.013
<i>CYP2C19</i> *17/*17	14	0.051	11	0.035	15	0.049
<i>CYP2C19</i> *2/*17	20	0.073	23	0.074	24	0.078
<i>Allele</i>	Number of alleles	Frequency	Number of alleles	Frequency	Number of alleles	Frequency
<i>CYP2C19</i> *1	358	0.649	410	0.659	388	0.628
<i>CYP2C19</i> *2	83	0.150	116	0.187	94	0.152
<i>CYP2C19</i> *17	111	0.201	96	0.154	136	0.220



**Table 3**

Frequency of the 10 haplotypes inferred in the entire study of 896 subjects.

Haplotype No.	<i>CYP2C19*17</i>	<i>CYP2C19*2</i>	<i>CYP2C9*2</i>	<i>CYP2C9*3</i>	<i>CYP2C8*3</i>	Number of haplotypes	PHASE frequency	STATA frequency
<b>1</b>	-	-	-	-	-	869	0.4862	0.4866
<b>2</b>	√	-	-	-	-	342	0.1901	0.1902
<b>3</b>	-	√	-	-	-	286	0.1589	0.1595
<b>4</b>	-	-	√	-	-	39	0.0218	0.0219
<b>5</b>	-	-	-	√	-	102	0.0562	0.0558
<b>6</b>	-	-	-	-	√	4	0.0023	0.0023
<b>7</b>	√	-	-	-	√	1	0.0006	8.3E-30
<b>8</b>	-	√	-	-	√	5	0.0026	0.0027
<b>9</b>	-	-	√	-	√	142	0.0787	0.0791
<b>10</b>	-	√	√	-	√	2	0.0012	0.0001